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    FILE 'MEDLINE, EMBASE, BIOSIS' ENTERED AT 12:43:57 ON 13 MAY 2005
L1         75020 S PLASMODIUM
L2          6213 S KOCKEN?/AU OR HOLDER?/AU OR WITHERS-MARTINEZ?/AU OR HENDRICUS
L3         52477 S FALCIPARUM OR (FALCIPARUM (P) FVO)
L4           570 S "APICAL MEMBRANE ANTIGEN" OR AMA1 OR AMA-1
L5        286010 S YEAST OR PASTORIS OR PICHIA
L6         32991 S GLYCOSYLATION (P) (PROTEIN OR PEPTIDE)
L7           197 S  RICH (2W) ("A+T" OR "A-T" OR "A/T")
L8            44 S L2 AND L1 AND L4
L9            16 S L8 NOT PY>=2002
L10            6 DUP REM L9 (10 DUPLICATES REMOVED)
L11            0 S L6 AND L4 AND L7
L12         5022 S "CODON USAGE" OR "CODON OPTIMIZATION"
L13            0 S "CODOP"
L14          115 S L12 AND L1
L15            3 S L14 AND L4
L16            1 DUP REM L15 (2 DUPLICATES REMOVED)
L17            9 S L14 AND L5
L18            3 S L17 NOT PY>=2002
L19            1 DUP REM L18 (2 DUPLICATES REMOVED)
L20           18 S L5 AND L3 AND L2
L21           10 S L20 NOT PY>=2002
L22            4 DUP REM L21 (6 DUPLICATES REMOVED)
L23            0 S L4 AND L7
L24            0 S L1 AND L7
L25            0 S L2 AND L7
L26          364 S  HIGH (2W) ("A+T" OR "A-T" OR "A/T")
L27           21 S L26 AND L1
L28            0 S L27 AND L4
L29           14 S L27 NOT PY>=2002
L30            6 DUP REM L29 (8 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001454963 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11399764
TITLE: Proteolytic processing and primary structure of **Plasmodium** falciparum **apical membrane antigen**-1.
AUTHOR: Howell S A; **Withers-Martinez C; Kocken C H;** Thomas A W; Blackman M J
CORPORATE SOURCE: Division of Protein Structure and the Division of Parasitology, National Institute for Medical Research, Mill Hill, London NW7 1AA, United Kingdom.
SOURCE: Journal of biological chemistry, (2001 Aug 17) 276 (33) 31311-20. Electronic Publication: 2001-06-08. Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010814
Last Updated on STN: 20030105
Entered Medline: 20010906

AB **Plasmodium** falciparum **apical membrane antigen**-1 (PfAMA-1) is a malaria merozoite integral membrane protein that plays an essential but poorly understood role in invasion of host erythrocytes. The PfAMA-1 ectodomain comprises three disulfide-constrained domains, the first of which (domain I) is preceded by an N-terminal prosequence. PfAMA-1 is initially routed to secretory organelles at the apical end of the merozoite, where the 83-kDa precursor (PfAMA-1(83)) is converted to a 66-kDa form (PfAMA-1(66)). At about the time of erythrocyte invasion, PfAMA-1(66) selectively translocates onto the merozoite surface. Here we use direct microsequencing and mass spectrometric peptide mass fingerprinting to characterize in detail the primary structure and proteolytic processing of PfAMA-1. We have determined the site at which processing takes place to convert PfAMA-1(83) to PfAMA-1(66) and have shown that both species possess a completely intact and unmodified transmembrane and cytoplasmic domain. Following relocation to the merozoite surface, PfAMA-1(66) is further proteolytically cleaved at one of two alternative sites, either between domains II and III, or at a membrane-proximal site following domain III. As a result, the bulk of the ectodomain is shed from the parasite surface in the form of two soluble fragments of 44 and 48 kDa. PfAMA-1 is not detectably modified by the addition of N-linked oligosaccharides.

L10 ANSWER 2 OF 6 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000231832 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10768987
TITLE: Immunization with parasite-derived **apical membrane antigen** 1 or passive immunization with a specific monoclonal antibody protects BALB/c mice against lethal **Plasmodium** yoelii yoelii YM blood-stage infection.
AUTHOR: Narum D L; Ogun S A; Thomas A W; **Holder A A**
CORPORATE SOURCE: Division of Parasitology, National Institute for Medical Research, London, NW7 1AA, United Kingdom.. davidn@entremed.com
SOURCE: Infection and immunity, (2000 May) 68 (5) 2899-906. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000622
Last Updated on STN: 20000622
Entered Medline: 20000613

AB We have purified apical merozoite antigen 1 (**AMA-1**) from extracts of red blood cells infected with the rodent malaria parasite

Plasmodium yoelii yoelii YM. When used to immunize mice, the protein induced a strong protective response against a challenge with the parasite. Monoclonal antibodies specific for P. yoelii yoelii **AMA -1** were prepared, and one was very effective against the parasite on passive immunization. A second protein that appears to be located in the apical rhoptry organelles and associated with **AMA -1** was identified.

L10 ANSWER 3 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000497406 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10960173
TITLE: Molecular characterisation of **Plasmodium** reichenowi **apical membrane antigen**-1 (**AMA-1**), comparison with P. falciparum **AMA-1**, and antibody-mediated inhibition of red cell invasion.
AUTHOR: **Kocken C H**; Narum1 D L; Massougbodji A; Ayivi B; Dubbeld M A; van der Wel A; Conway D J; Sanni A; Thomas A W
CORPORATE SOURCE: Biomedical Primate Research Centre, Department of Parasitology, Rijswijk, The Netherlands.
SOURCE: Molecular and biochemical parasitology, (2000 Jul) 109 (2) 147-56.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ252087; GENBANK-AJ271168; GENBANK-AJ271190
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001019

AB **Apical membrane antigen** 1 is a candidate vaccine component for malaria. It is encoded by a single copy gene and has been characterised in a number of malaria species as either an 83-kDa de novo product (**Plasmodium** falciparum; Pf **AMA-1**) or a 66-kDa product (all other species). All members of the **AMA-1** family are expressed during merozoite formation in maturing schizonts and are initially routed to the rhoptries. Processed forms may subsequently be associated with the merozoite surface. Because of the unique occurrence of the 83-kDa form in P. falciparum we were interested to determine whether the phylogenetically closely related chimpanzee malaria **Plasmodium** reichenowi shared characteristics with Pf **AMA-1**. Here we show that the molecular structure, the localisation and processing are similar to that of Pf **AMA-1** and that in vitro growth inhibitory mAbs reactive with Pf **AMA-1** also inhibit P. reichenowi growth in an in vitro assay. Polymorphism in the 83-kDa **AMA-1** family was analysed through comparison of Pr **ama-1** with Pf **ama-1** alleles, which showed the most significant evidence for selection maintaining polymorphism in Domains I-III of **AMA-1** in P. falciparum. The most substantial divergence between Pr **AMA-1** and Pf **AMA-1** sequences was in the N-terminal region unique to the 83-kDa form of **AMA-1**. It was confirmed that the specific Pr **ama-1**-type allele was not present among P. falciparum parasites in an African population, and an allele coding for lysine at amino acid 187 was uniquely associated with field isolates in this population.

L10 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 1999081721 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9864194
TITLE: High-level expression of **Plasmodium** vivax **apical membrane antigen** 1 (**AMA-1**) in Pichia pastoris: strong immunogenicity in Macaca mulatta immunized with P. vivax **AMA-1** and adjuvant SBAS2.
AUTHOR: **Kocken C H**; Dubbeld M A; Van Der Wel A; Pronk J

T; Waters A P; Langermans J A; Thomas A W
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, The Netherlands.
SOURCE: Infection and immunity, (1999 Jan) 67 (1) 43-9.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Y16950
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 19990209
Entered Medline: 19990128

AB The **apical membrane antigen** 1 (**AMA-1**) family is a promising family of malaria blood-stage vaccine candidates that have induced protection in rodent and nonhuman primate models of malaria. Correct conformation of the protein appears to be essential for the induction of parasite-inhibitory responses, and these responses appear to be primarily antibody mediated. Here we describe for the first time high-level secreted expression (over 50 mg/liter) of the **Plasmodium** vivax **AMA-1** (PV66/**AMA-1**) ectodomain by using the methylotrophic yeast Pichia pastoris. To prevent nonnative glycosylation, a conservatively mutagenized PV66/**AMA-1** gene (PV66Deltaglyc) lacking N-glycosylation sites was also developed. Expression of the PV66Deltaglyc ectodomain yielded similar levels of a homogeneous product that was nonglycosylated and was readily purified by ion-exchange and gel filtration chromatographies. Recombinant PV66Deltaglyc43-487 was reactive with conformation-dependent monoclonal antibodies. With the SBAS2 adjuvant, Pichia-expressed PV66Deltaglyc43-487 was highly immunogenic in five rhesus monkeys, inducing immunoglobulin G enzyme-linked immunosorbent assay titers in excess of 1:200,000. This group of monkeys had a weak trend showing lower cumulative parasite loads following a **Plasmodium** cynomolgi infection than in the control group.

L10 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 1998279031 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9614123
TITLE: Precise timing of expression of a **Plasmodium** falciparum-derived transgene in **Plasmodium** berghei is a critical determinant of subsequent subcellular localization.
AUTHOR: **Kocken C H**; van der Wel A M; Dubbeld M A; Narum D L; van de Rijke F M; van Gemert G J; van der Linde X; Bannister L H; Janse C; Waters A P; Thomas A W
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research Centre, Lange Kleiweg 157, 2280 GJ Rijswijk, The Netherlands.
SOURCE: Journal of biological chemistry, (1998 Jun 12) 273 (24) 15119-24.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980723
Last Updated on STN: 19980723
Entered Medline: 19980713

AB The development of transfection technology for malaria parasites holds significant promise for a more detailed characterization of molecules targeted by vaccines or drugs. One asexual blood stage vaccine candidate, **apical membrane antigen**-1 (**AMA-1**) of merozoite rhoptries has been shown to be the target of inhibitory, protective antibodies in both in vitro and in vivo studies. We have investigated heterologous (trans-species) expression of the human malaria **Plasmodium** falciparum **AMA-1** (PF83/**AMA-1**) in the rodent parasite **Plasmodium**

berghei. Transfected P. berghei expressed correctly folded and processed PF83/**AMA-1** under control of both pb66/**ama-1** and dhfr-ts promoters. Timing of expression was highly promoter-dependent and was critical for subsequent subcellular localization. Under control of pb66/**ama-1**, PF83/**AMA-1** expression and localization in P. berghei was limited to the rhoptries of mature schizonts, similar to that observed for PF83/**AMA-1** in P. falciparum. In contrast the dhfr-ts promoter permitted PF83/**AMA-1** expression throughout schizogony as well as in gametocytes and gametes. Localization was aberrant and included direct expression at the merozoite and gamete surface. Processing from the full-length 83-kDa protein to a 66-kDa protein was observed not only in schizonts but also in gametocytes, indicating that processing could be mediated outside of rhoptries by a common protease. Trans-species expressed PF83/**AMA-1** was highly immunogenic in mice, resulting in a response against a functionally critical domain of the molecule.

L10 ANSWER 6 OF 6 MEDLINE on STN
ACCESSION NUMBER: 96299160 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8660634
TITLE: Rapid screening and mapping of conformational epitopes expressed in the secretion expression system Pichia pastoris.
AUTHOR: **Kocken C H**; Thomas A W
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research Centre, Rijswijk, 2280 GH, The Netherlands.
SOURCE: Analytical biochemistry, (1996 Jul 15) 239 (1) 111-2. Journal code: 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 19961025
Entered Medline: 19961017

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L16 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2002372320 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12117958
TITLE: High-level expression of the malaria blood-stage vaccine candidate **Plasmodium** falciparum **apical membrane antigen** 1 and induction of antibodies that inhibit erythrocyte invasion.
COMMENT: Erratum in: Infect Immun 2002 Oct;70(10):5901
AUTHOR: Kocken Clemens H M; Withers-Martinez Chrislaine; Dubbeld Martin A; van der Wel Annemarie; Hackett Fiona; Valderrama Augusto; Blackman Michael J; Thomas Alan W
CORPORATE SOURCE: Department of Parasitology, Biomedical Primate Research Centre, 2280 GH Rijswijk, The Netherlands.
SOURCE: Infection and immunity, (2002 Aug) 70 (8) 4471-6.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ277646
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 20020716
Last Updated on STN: 20021022
Entered Medline: 20020904

AB **Apical membrane antigen** 1 (**AMA-1**) is a highly promising malaria blood-stage vaccine candidate that has induced protection in rodent and nonhuman primate models of malaria. Authentic conformation of the protein appears to be essential for the induction of parasite-inhibitory antibody responses. Here we have developed a synthetic gene with adapted **codon usage** to allow expression of **Plasmodium** falciparum FVO strain **AMA-1** (PfAMA-1) in Pichia pastoris. In addition, potential N-glycosylation sites were changed, exploiting the lack of conservation of these sites in **Plasmodium**, to obtain high-level secretion of a homogeneous product, suitable for scale-up according to current good manufacturing procedures. Purified PfAMA-1 displayed authentic antigenic properties, indicating that the amino acid changes had no deleterious effect on the conformation of the protein. High-titer antibodies, raised in rabbits, reacted strongly with homologous and heterologous P. falciparum by immunofluorescence. In addition, purified immunoglobulin G from immunized animals strongly inhibited invasion of red blood cells by homologous and, to a somewhat lesser extent, heterologous P. falciparum.

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L19 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2000079309 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10611405
TITLE: PCR-based gene synthesis as an efficient approach for expression of the A+T-rich malaria genome.
AUTHOR: Withers-Martinez C; Carpenter E P; Hackett F; Ely B; Sajid M; Grainger M; Blackman M J
CORPORATE SOURCE: Division of Parasitology, Division of Protein Structure, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.
SOURCE: Protein engineering, (1999 Dec) 12 (12) 1113-20.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ242589
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20010723
Entered Medline: 20000211

AB The A+T-rich genome of the human malaria parasite **Plasmodium** falciparum encodes genes of biological importance that cannot be expressed efficiently in heterologous eukaryotic systems, owing to an extremely biased **codon usage** and the presence of numerous cryptic polyadenylation sites. In this work we have optimized an assembly polymerase chain reaction (PCR) method for the fast and extremely accurate synthesis of a 2.1 kb **Plasmodium** falciparum gene (pfsub-1) encoding a subtilisin-like protease. A total of 104 oligonucleotides, designed with the aid of dedicated computer software, were assembled in a single-step PCR. The assembly was then further amplified by PCR to produce a synthetic gene which has been cloned and successfully expressed in both **Pichia pastoris** and recombinant baculovirus-infected High Five(TM) cells. We believe this strategy to be of special interest as it is simple, accessible and has no limitation with respect to the size of the gene to be synthesized. Used as a systematic approach for the malarial genome or any other A + T-rich organism, the method allows the rapid synthesis of a nucleotide sequence optimized for expression in the system of choice and production of sufficiently large amounts of biological material for complete molecular and structural characterization.

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L22 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2000079309 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10611405
TITLE: PCR-based gene synthesis as an efficient approach for expression of the A+T-rich malaria genome.
AUTHOR: **Withers-Martinez C**; Carpenter E P; Hackett F; Ely B; Sajid M; Grainger M; Blackman M J
CORPORATE SOURCE: Division of Parasitology, Division of Protein Structure, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.
SOURCE: Protein engineering, (1999 Dec) 12 (12) 1113-20. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ242589
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000229
Last Updated on STN: 20010723
Entered Medline: 20000211

AB The A+T-rich genome of the human malaria parasite Plasmodium **falciparum** encodes genes of biological importance that cannot be expressed efficiently in heterologous eukaryotic systems, owing to an extremely biased codon usage and the presence of numerous cryptic polyadenylation sites. In this work we have optimized an assembly polymerase chain reaction (PCR) method for the fast and extremely accurate synthesis of a 2.1 kb Plasmodium **falciparum** gene (pfsub-1) encoding a subtilisin-like protease. A total of 104 oligonucleotides, designed with the aid of dedicated computer software, were assembled in a single-step PCR. The assembly was then further amplified by PCR to produce a synthetic gene which has been cloned and successfully expressed in both **Pichia pastoris** and recombinant baculovirus-infected High Five(TM) cells. We believe this strategy to be of special interest as it is simple, accessible and has no limitation with respect to the size of the gene to be synthesized. Used as a systematic approach for the malarial genome or any other A + T-rich organism, the method allows the rapid synthesis of a nucleotide sequence optimized for expression in the system of choice and production of sufficiently large amounts of biological material for complete molecular and structural characterization.

L22 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1999272559 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10339410
TITLE: Solution structure of an EGF module pair from the Plasmodium **falciparum** merozoite surface protein 1.
AUTHOR: Morgan W D; Birdsall B; Frenkiel T A; Gradwell M G; Burghaus P A; Syed S E; Uthaipibull C; **Holder A A**; Feeney J
CORPORATE SOURCE: Molecular Structure Division, The Ridgeway Mill Hill, London, NW7 1AA, UK.
SOURCE: Journal of molecular biology, (1999 May 28) 289 (1) 113-22. Journal code: 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1CEJ; PDB-R1CEJMR
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 20000303
Entered Medline: 19990715

AB The solution structure of the 96-residue C-terminal fragment of the merozoite surface protein 1 (MSP-1) from Plasmodium **falciparum** has been determined using nuclear magnetic resonance (NMR) spectroscopic measurements on uniformly13C/15N-labelled protein, efficiently expressed

in the methylotrophic **yeast** Komagataella (**Pichia**) **pastoris**. The structure has two domains with epidermal growth factor (EGF)-like folds with a novel domain interface for the EGF domain pair interactions, formed from a cluster of hydrophobic residues. This gives the protein a U-shaped overall structure with the N-terminal proteolytic processing site close to the C-terminal glycosyl phosphatidyl inositol (GPI) membrane anchor site, which is consistent with the involvement of a membrane-bound proteinase in the processing of MSP-1 during erythrocyte invasion. This structure, which is the first protozoan EGF example to be determined, contrasts with the elongated structures seen for EGF-module pairs having shared Ca2+-ligation sites at their interface, as found, for example, in fibrillin-1. Recognition surfaces for antibodies that inhibit processing and invasion, and antibodies that block the binding of these inhibitory antibodies, have been mapped on the three-dimensional structure by considering specific MSP-1 mutants.

L22 ANSWER 3 OF 4 MEDLINE on STN
ACCESSION NUMBER: 97214043 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9060445
TITLE: Growth and storage of YAC clones in Hogness Freezing Medium.
AUTHOR: Werner E; **Holder A A**; Hoheisel J D
CORPORATE SOURCE: Molecular-Genetic Genome Analysis, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506, D-69120 Heidelberg, Germany.
SOURCE: Nucleic acids research, (1997 Apr 1) 25 (7) 1467-8. Journal code: 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970514
Last Updated on STN: 19980206
Entered Medline: 19970502

AB To date, frozen storage of YAC libraries have relied on the administration of glycerol to the medium subsequent to cell growth. By adding Hogness Freezing Medium prior to inoculation, cultures can be frozen directly after cell growth, with no adverse effect on the stability of the YAC DNA or on the viability of the cells even after repeated freezing and defrosting. Although a relatively simple modification, the procedure notably improves the handling of YAC libraries and significantly reduces the risk of contamination, especially when dealing with large numbers of clones.

L22 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 97001675 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8844667
TITLE: Current status of the Plasmodium **falciparum** genome project.
AUTHOR: Dame J B; Arnot D E; Bourke P F; Chakrabarti D; Christodoulou Z; Coppel R L; Cowman A F; Craig A G; Fischer K; Foster J; Goodman N; Hinterberg K; **Holder A A**; Holt D C; Kemp D J; Lanzer M; Lim A; Newbold C I; Ravetch J V; Reddy G R; Rubio J; Schuster S M; Su X Z; Thompson J K; Werner E B; +
CORPORATE SOURCE: University of Florida, Gainesville, 32611, USA.. dame@icbr.ifas.ufl.edu
SOURCE: Molecular and biochemical parasitology, (1996 Jul) 79 (1) 1-12. Ref: 74
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(MULTICENTER STUDY)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AA549841; GENBANK-AA549842; GENBANK-AA549843;
GENBANK-AA549844; GENBANK-AA549845; GENBANK-AA549846;
GENBANK-AA549847; GENBANK-AA549848; GENBANK-AA549849;
GENBANK-AA549850; GENBANK-AA549851; GENBANK-AA549852;
GENBANK-AA549853; GENBANK-AA549854; GENBANK-AA549855;
GENBANK-AA549856; GENBANK-AA549857; GENBANK-AA549858;
GENBANK-AA549859; GENBANK-AA549860; GENBANK-AA549861;
GENBANK-AA549862; GENBANK-AA549863; GENBANK-AA549864;
GENBANK-AA549865; GENBANK-AA549866; GENBANK-AA549867
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 20000303
Entered Medline: 19970129

AB The Plasmodium **falciparum** Genome Project is a collaborative effort by many laboratories that will provide detailed molecular information about the parasite, which may be used for developing practical control measures. Initial goals are to prepare an electronically indexed clone bank containing partially sequenced clones representing up to 80% of the parasite's genes and to prepare an ordered set of overlapping clones spanning each of the parasite's 14 chromosomes. Currently, clones of genomic DNA, prepared as **yeast** artificial chromosomes, are arranged into contigs covering approximately 70% of the genome of parasite clone 3D7, gene sequence tags are available from more than contigs covering approximately 70% of the genome of parasite clone 3D7, gene sequence tags are available from more than 20% of the parasite's genes, and approximately 5% of the parasite's genes are tentatively identified from similarity searches of entries in the international sequence databases. A total of > 0.5 Mb of P. **falciparum** sequence tag data is available. The gene sequence tags are presently being used to complete YAC contig assembly and localize the cloned genes to positions on

L30 ANSWER 1 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2002180006 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11913781
TITLE: M13 cloning of mung bean nuclease digested PCR fragments as a means of gap closure within A/T-rich, genome sequencing projects.
AUTHOR: Quail M A
CORPORATE SOURCE: The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK.. mq1@sanger.ac.uk
SOURCE: DNA sequence : journal of DNA sequencing and mapping, (2001 Dec) 12 (5-6) 355-9.
Journal code: 9107800. ISSN: 1042-5179.
PUB. COUNTRY: Switzerland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020401
Last Updated on STN: 20020815
Entered Medline: 20020814

AB Obtaining the complete DNA sequence of a genome is often not straightforward. After standard shotgun sequencing strategies have been employed there are often gaps remaining and these can be the most intractable regions, frequently containing repeat sequences, "uncloneable" sequences and/or regions of potential secondary structure or differential base composition. In genomes with a **high A/T** content, such as **Plasmodium** falciparum and Dictyostelium discoideum, solving these gaps is a particularly difficult problem as the sequences concerned are "fragile" and easily denatured, commonly uncloneable and have a paucity of good oligonucleotide priming sites. Reported here is a simple, yet reliable method for determining the sequence of A/T-rich gap-spanning PCR products. This method relies on the slippage of the specificity of mung bean nuclease so that it digests A/T-rich double-stranded DNA into a set of deletion fragments that can then be cloned into M13, sequenced and the original sequence assembled therefrom.

L30 ANSWER 2 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001324188 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11254951
TITLE: Serial analysis of gene expression (SAGE) in **Plasmodium** falciparum: application of the technique to A-T rich genomes.
AUTHOR: Munasinghe A; Patankar S; Cook B P; Madden S L; Martin R K; Kyle D E; Shoaibi A; Cummings L M; Wirth D F
CORPORATE SOURCE: Department of Immunology and Infectious Diseases, Harvard School of Public Health, Harvard University, Building 1, Room 704, 665 Huntington Ave, Boston MA 02115, USA.
SOURCE: Molecular and biochemical parasitology, (2001 Mar) 113 (1) 23-34.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010611
Last Updated on STN: 20010611
Entered Medline: 20010607

AB The advent of high-throughput methods for the analysis of global gene expression, together with the Malaria Genome Project open up new opportunities for furthering our understanding of the fundamental biology and virulence of the malaria parasite. Serial analysis of gene expression (SAGE) is particularly well suited for malarial systems, as the genomes of **Plasmodium** species remain to be fully annotated. By simultaneously and quantitatively analyzing mRNA transcript profiles from a given cell population, SAGE allows for the discovery of new genes. In this study, one reports the successful application of SAGE in

Plasmodium falciparum, 3D7 strain parasites, from which a preliminary library of 6880 tags corresponding to 4146 different genes was generated. It was demonstrated that P. falciparum is amenable to this technique, despite the remarkably **high A-T** content of its genome. SAGE tags as short as 10 nucleotides were sufficient to uniquely identify parasite transcripts from both nuclear and mitochondrial genomes. Moreover, the skewed A-T content of parasite sequence did not preclude the use of enzymes that are crucial for generating representative SAGE libraries. Finally, a few modifications to DNA extraction and cloning steps of the SAGE protocol proved useful for circumventing specific problems presented by A-T rich genomes.

L30 ANSWER 3 OF 6 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 96081463 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8520581
TITLE: Phylogeny of the large extrachromosomal DNA of organisms in the phylum Apicomplexa.
COMMENT: Erratum in: J Eukaryot Microbiol. 1996 Mar-Apr;43(2):158. PubMed ID: 8720946
AUTHOR: Egea N; Lang-Unnasch N
CORPORATE SOURCE: Department of Medicine, University of Alabama at Birmingham 35294-2170, USA.
CONTRACT NUMBER: AI 28780 (NIAID)
SOURCE: Journal of eukaryotic microbiology, (1995 Nov-Dec) 42 (6) 679-84.
Journal code: 9306405. ISSN: 1066-5234.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U28056
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 19980206
Entered Medline: 19960122

AB Organisms in the phylum Apicomplexa appear to have a large extrachromosomal DNA which is unrelated to the mitochondrial DNA. Based on the apparent gene content of the large (35 kb) extrachromosomal DNA of **Plasmodium** falciparum, it has been suggested that it is a plastid-like DNA, which may be related to the plastid DNA of rhodophytes. However, phylogenetic analyses have been inconclusive. It has been suggested that this is due to the unusually **high A+T** content of the **Plasmodium** falciparum large extrachromosomal DNA. To further investigate the evolution of the apicomplexan large extrachromosomal DNA, the DNA sequence of the organellar ribosomal RNA gene from Toxoplasma gondii, was determined. The Toxoplasma gondii rDNA sequence was most similar to the large extrachromosomal rDNA of **Plasmodium** falciparum, but was much less A+T rich. Phylogenetic analyses were carried out using the LogDet transformation to minimize the impact of nucleotide bias. These studies support the evolutionary relatedness of the Toxoplasma gondii rDNA with the large extrachromosomal rDNA of **Plasmodium** falciparum and with the organellar rDNA of another parasite in the phylum Apicomplexa, Babesia bovis. These analyses also suggest that the apicomplexan large extrachromosomal DNA may be more closely related to the plastid DNA of euglenoids than those of rhodophytes.

L30 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 93173202 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8437626
TITLE: Transcriptional differences in polymorphic and conserved domains of a complete cloned P. falciparum chromosome.
AUTHOR: Lanzer M; de Bruin D; Ravetch J V
CORPORATE SOURCE: DeWitt Wallace Research Laboratory, Sloan-Kettering Institute, Division of Molecular Biology, New York, New York 10021.
SOURCE: Nature, (1993 Feb 18) 361 (6413) 654-7.
Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199303
ENTRY DATE: Entered STN: 19930402
Last Updated on STN: 19930402
Entered Medline: 19930322

AB Classical genetic studies on the human malaria parasite **Plasmodium** falciparum have been hampered by a complex life cycle which alternates between vertebrate and invertebrate hosts. Consequently, only a few genetic crosses have been performed so far. In addition, molecular genetics has provided only limited access to the genes of this pathogen, a consequence of an unusually **high A + T** content. To overcome these limitations we have constructed an ordered telomere-to-telomere contig map of P. falciparum chromosome 2 by isolating overlapping yeast artificial chromosome clones. This approach was used to examine the strain-dependent polymorphisms commonly observed for P. falciparum chromosomes. Our analysis reveals that polymorphisms of chromosome 2 are restricted to regions at either end, representing 20% of the chromosome. Transcription mapping of the entire chromosome suggests a compartmentalization of chromosome 2 into a transcribed central domain and silent polymorphic ends.

L30 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 89364996 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2671721
TITLE: Stage-specific expression and genomic organization of the actin genes of the malaria parasite **Plasmodium** falciparum.
AUTHOR: Wesseling J G; Snijders P J; van Someren P; Jansen J; Smits M A; Schoenmakers J G
CORPORATE SOURCE: Department of Molecular Biology, University of Nijmegen, The Netherlands.
SOURCE: Molecular and biochemical parasitology, (1989 Jun 15) 35 (2) 167-76.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J03988; GENBANK-M22718; GENBANK-M22719
ENTRY MONTH: 198910
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19891012

AB Two different actin transcripts are found in the human malaria parasite **Plasmodium** falciparum. One of these is a 2.5-kb-long RNA found both in asexual blood stages and in the sexual stages (i.e., gametes/zygotes) of the parasite. This transcript is encoded by the P. falciparum (pf)-actin I gene. The second malarial actin gene, the pf-actin II gene, yields a 1.9-kb-long transcript which is formed solely in the sexual stages. Elucidation of the genomic organisation of these two **Plasmodium** actin genes showed that the pf-actin I gene does not possess any introns whereas the coding region of the pf-actin II gene is interrupted by a 368-bp intron. This intron has consensus splice junction sequences. Nucleotide sequence analysis of the 3' non-coding regions of the pf-actin genes revealed that these regions are quite long (pf-actin I, 250 bp; pf-actin II, 331 bp) and that these trailers do not share sequence similarity. Furthermore, the poly(A)+ addition sites of both actin mRNAs have now been identified. The 5' untranslated regions are also rather long; the sequenced areas lack sequence similarity and have, as do the 3' untranslated regions, a very **high A + T** content.

L30 ANSWER 6 OF 6 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 88068594 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2825189
TITLE: Molecular cloning and sequence analysis of the **Plasmodium** falciparum dihydrofolate

reductase-thymidylate synthase gene.
AUTHOR: Bzik D J; Li W B; Horii T; Inselburg J
CORPORATE SOURCE: Department of Microbiology, Dartmouth Medical School, Hanover, NH 03756.
CONTRACT NUMBER: AI 20437 (NIAID)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1987 Dec) 84 (23) 8360-4. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J03028
ENTRY MONTH: 198801
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19980206
Entered Medline: 19880107

AB Genomic DNA clones that coded for the bifunctional dihydrofolate reductase (DHFR) and thymidylate synthase (TS) (DHFR-TS) activities from a pyrimethamine-sensitive strain of **Plasmodium** falciparum were isolated and sequenced. The deduced DHFR-TS protein contained 608 amino acids (71,682 Da). The coding region for DHFR-TS contained no intervening sequences and had a **high A** + **T** content (75%). The DHFR domain, in the amino-terminal portion of the protein, was joined by a 94-amino acid junction sequence to the TS domain in the carboxyl-terminal portion of the protein. The TS domain was more conserved than the DHFR domain and both P. falciparum domains were more homologous to eukaryotic than to prokaryotic forms of the enzymes. Predicted secondary structures of the DHFR and TS domains were nearly identical to the structures identified in other DHFR and TS enzymes.

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